Anti-phospholipid/Cofactor Antibodies

Eric Yuk-Tat Chan

Abstract: There has been considerable uncertainty about the criteria for the diagnosis of anti-phospholipid syndrome (APS). Apart from the classical clinical features of vascular thrombosis and recurrent abortions, a long list of other clinical manifestations has been attributed to this syndrome. In addition to traditional laboratory tests such as the anti-cardiolipin antibody and lupus anti-coagulant assays, a number of other tests are also available for the diagnosis of APS because of the inadequacy of current tests. Among these, anti-β2-glycoprotein I antibody appears to be specific for the diagnosis of APS but requires more studies to confirm.

Keywords: Anti-β2-glycoprotein I antibody, anti-cardiolipin antibody, anti-phospholipid syndrome

Diagnosis of Anti-phospholipid Syndrome

Following a meeting at Sapporo, Japan, an international consensus statement on preliminary classification criteria for definite anti-phospholipid syndrome was published.¹ This set of criteria is divided into the clinical part which includes vascular thrombosis and pregnancy morbidity and the laboratory part which includes anti-cardiolipin (aCL) antibody and lupus anti-coagulant tests. Diagnosis of APS is considered definite if a patient has at least 1 clinical and 1 laboratory criteria. The latter should be positive for at least 2 times 6 weeks apart so that transient occurrence of anti-phospholipid antibodies are not included.

A number of "minor" criteria have been discussed but not included in the Sapporo criteria because there is insufficient evidence to support their diagnostic values. Examples are transient ischaemia attack, transverse myelopathy or myelitis, chorea, migraine, livedo reticularis, heart valve lesions, adrenal hemorrhage, thrombocytopenia, haemolytic anaemia and laboratory tests such as anti-β2-glycoprotein I (GPI), IgA aCL, low IgG & IgM aCL, VDRL, antibodies to other phospholipids. Validation study of the Sapporo criteria for APS showed a diagnostic sensitivity of 71% (negative predictive value 88%) and a diagnostic specificity of 98% (positive predictive value 95%).² Most of the false-negative cases were due to the "minor" criteria although some were truly seronegative.

Anti-cardiolipin Antibodies

Cardiolipin is the most common antigen used in solid phase assay to detect anti-phospholipid antibodies. The basic structure of cardiolipin consists of 2 phospholipids each composed of a substituted group linked by a phosphodiester bond to glyceride. Most anti-phospholipid antibodies bind epitopes on the phosphodiester group. Anti-cardiolipin antibodies are therefore a heterogenous group of antibodies. Anti-cardiolipin antibody is measured by indirect antibody ELISA (Enzyme-linked Immunosorbent Assay). In this technique, the antigen (cardiolipin) is used to coat the ELISA plate, test sera are added and bound antibodies are detected by enzyme conjugated anti-human Ig. Both cardiolipin and enzyme conjugates are available commercially and one can easily develope an in-house assay. Guidelines to the detection of aCL antibodies have been published by the Association of Clinical Pathologists.³

β2-glycoprotein I in aCL ELISA

Addition of bovine serum to serum/conjugate diluent is a necessary step for aCL antibody detection. A constituent
protein, β2-glycoprotein I (GPI), is responsible for this enhancing phenomenon but the mechanism remains unclear. Possibilities that have been suggested are: (a) β2-GPI alters the conformation of cardiolipin resulting in better binding; (b) β2-GPI together with cardiolipin form the epitope for binding; (c) β2-GPI is the actual target in the antibody assay.

**aCL ELISA Problems**

It has been difficult to maintain good precision and accuracy for aCL ELISA and very often intra- and inter-laboratory assay differences are significant. For the latter, improvement can be made with the availability of international standard sera and the grading of numerical results. Using the international standard sera for calibration, results are expressed in GPL unit/ml or MPL unit/ml for IgG and IgM aCL respectively. One GPL or MPL unit/ml is defined as the binding activity of 1 mg/ml of IgG or IgM standard serum respectively. It is also recommended that numerical results should be graded into negative, weak, moderate, strong. These semi-quantitative results have a much better correlation among different laboratories. The cutoff between negative and weakly positive is determined using a batch of normal sera. The cutoff between weakly and moderately positive is usually 10 units above the negative/weak cutoff. The cutoff between moderately and strongly positive is usually 50 MPL/ml and 80 GPL/ml for IgM and IgG aCL respectively. Accordingly the ranges for the aCL results currently performed in the Immunology laboratory of the Queen Mary Hospital will be:

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<tr>
<th></th>
<th>Negative</th>
<th>Weak</th>
<th>Moderate</th>
<th>Strong</th>
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</thead>
<tbody>
<tr>
<td>IgM (MPL/ml)</td>
<td>&lt;13</td>
<td>13-23</td>
<td>23-50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>IgG (GPL/ml)</td>
<td>&lt;15</td>
<td>15-25</td>
<td>25-80</td>
<td>&gt;80</td>
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These grades are used in quality assurance program reports which demonstrate good correlation between laboratories if a one-grade difference is considered acceptable. These reference ranges should be evaluated for their prediction of clinical thrombosis locally.

**Clinical Significance of aCL Isotypes**

IgG aCL is most related to the clinical complications of anti-phospholipid syndrome. The value of IgM aCL is more doubtful since transient positive cases are more commonly of the IgM isotype. In addition, false positive IgM aCL results may be due to the presence of rheumatoid factor in serum. IgA aCL has been undisputely discarded for a long time.

**Anti-β2-glycoprotein I (GPI)**

β2-GPI is first described in 1961 as a perchloric acid soluble human plasma protein. It is also called apolipoprotein H and is synthesized in the liver as a single-chain highly glycosylated polypeptide with a molecular weight of 50 kD. β2-GPI has a highly cationic C-terminal domain which binds to anionic phospholipids. It has *in vitro* anti-coagulant properties of inhibiting the activation of the contact phase of the intrinsic coagulation pathway. The *in vivo* functions are not clear but may be involved in lipid metabolism and coagulation.

**Clinical Value of Measuring Anti-β2GPI**

Several reports, including our preliminary data, claimed measurement of anti-β2-GPI is more specific for the diagnosis of APS than aCL. Others still think aCL is more clinically relevant and there is yet no recommendation that aCL should be replaced or supplemented by anti-β2-GPI.

The clinical value of β2-GPI and anti-β2-GPI may be related to their possible role in the pathogenesis of APS through increased tissue factor on cell surface which leads to physiologic coagulation or pathologic thrombosis. Increased tissue factor may be induced by inflammatory cytokines or by autoantibodies or immune complexes which involve anti-β2-GPI. Direct evidence of the latter is that monoclonal antibodies against β2-GPI and endothelial cells derived from patients with APS and vasculitis can increase tissue factor activity, antigen expression and mRNA expression. The increase is dose and time dependent, involves the F(ab)₂ but not the Fc part of the monoclonal antibodies and is inhibited by pre-treatment with anti-tissue factor antibodies.

**VDRL (Venereal Disease Research Laboratory) Test**

VDRL is a flocculation reaction. Like Wassermann reaction, which is a complement fixation test, VDRL detect reagin. The antigenic material used which is non-treponemal includes anionic phospholipid (cardiolipin), neutral phospholipid (phosphatidylcholine) and cholesterol. False positive results of VDRL may therefore be due to presence of anti-cardiolipin antibody. This is reported in 20-30% of patients with APS or SLE. Sera from patients with syphilis differ from sera from patients with APS/SLE in several ways. First syphilis sera
bind both anionic and neutral phospholipid but APS/SLE sera bind only anionic phospholipid. In addition, syphilis sera give negative results with lupus anti-coagulant test and weakly positive results with aCL test (if positive) that are not β2-GPI dependent. The reverse is true for APS/SLE sera.

**Other Anionic Phospholipids**

Anionic phospholipids other than cardiolipin such as phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI) or phospholipids with no net charge such as phosphatidylcholine (PC), sphingomyelin, phosphatidylethanolamine (PE) may be used as alternative targets of autoantibody tests in APS. Such tests are developed because of the relatively low specificity of the aCL test for APS. A few studies did show a better clinical correlation with APS than aCL but most reported generally good correlation between tests using cardiolipin and other phospholipids and there is yet no definite evidence that the use of other phospholipids has better or additional clinical value.

**References**